

## Chapter 12

# Biofloc-based Aquaculture Systems

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Interest in biofloc-based aquaculture systems has grown over the past twenty years, because these systems can provide comparatively biosecure, more environmentally benign, and financially sustainable aquaculture production. Tilapia and shrimp are well suited to take advantage of natural productivity in aquaculture systems. Each currently represents quickly expanding commercial industries worldwide. Aquaculture production of an important tilapia species, *Oreochromis niloticus*, more than doubled between 2001 and 2007 from 1,030,888 tonnes to 2,121,009 tonnes (FAO 2010). Production of the most commonly cultured marine shrimp, *Litopenaeus vannamei*, more than doubled from 982,663 tonnes in 2003 to 2,296,630 tonnes in 2007 (FAO 2010). An increasing body of research is being devoted to development of biofloc-based applications for these species.

Biofloc technologies can be applied in ponds, tanks, or raceways of various scales. Intensive, biofloc-based production of shrimp is done in lined ponds, from 500 to 20,000 m<sup>2</sup>, with a seasonal production of 10 to 20 tons/ha (1 to 2 kg/m<sup>2</sup>). These systems are becoming quite common and are expanding quickly. Production of tilapia using biofloc systems in general produces a much higher biomass than that found in intensive shrimp ponds, in the range of 10 to 30 kg/m<sup>2</sup>. A third application of the technology targets super-intensive production of shrimp in tanks or raceways. Production levels in these systems can reach close to 10 kg/m<sup>2</sup>. The present chapter provides a review of the

characteristics of these biofloc systems focusing on the commonalities and highlighting management strategies that can differ according to application.

Biofloc systems are based on the concept of cultivating a microbial community within the production unit. This microbial community provides important ecosystem services including the cycling of waste material and provision of supplemental nutrition to the target crop. External inputs include the feed and in many cases supplemental carbon and/or bicarbonate to support target crop growth and to meet the needs of the microbial community. Inputs also include energy for supplemental aeration or oxygenation and mixing to maintain a suspended aerobic microbial consortium. Through proper management of the inputs, target crop density, and cropping or oxidation of organic material during and/or between crops, the grower can achieve a balance, thereby maximizing ecosystem services within the production unit. This can provide for improved cost efficiencies, stable production conditions, and higher overall environmental sustainability of production.

For centuries, fertilized ponds have provided a basis for artisanal production of fish and shrimp. As early as the late 1970s, a group in Israel studied the dynamics of fish culture systems enriched with organic material, developing the concept of a heterotrophic food web (Wohlfarth & Schroeder 1979; Hepher 1985). At the same time, Steven Serfling and Dominick Mendola conceptualized and developed a business based on industrial production of tilapia and shrimp in systems with dense microbial communities with reduced reliance on water exchange. Applying a more holistic approach, the systems focused on maximizing benefits from natural productivity, which developed in the systems. In their early research and commercialization efforts at Solar Aquafarms, some of the concepts that today characterize biofloc technologies were demonstrated, although they never reached the popular or scientific literature (Rosenberry 2007).

In the early 1990s, two groups working independently in Israel at the Technion University and in the United States at the Waddell Mariculture Center (WMC) began to publish a series of papers on the application of reduced and then zero exchange production technologies for tilapia and shrimp, respectively (Avnimelech 1993; Hopkins *et al.* 1993; Avnimelech *et al.* 1994; Hopkins *et al.* 1995b). The research demonstrated the assimilation of excess nitrogen into microbial biomass and its mineralization through nitrification and denitrification processes. At about the same time, a series of studies at the Oceanic Institute in Hawaii reported on the growth enhancement effects of factors in pond water from intensive culture systems (Leber & Pruder 1998; Moss 1995; Moss & Pruder 1995). Commercial farms began to adopt and refine these technologies, and shrimp operations such as Belize Aquaculture confirmed the commercial scale production potential (Boyd & Clay 2002). This increasing body of literature illustrated opportunities for intensification of production while reducing water exchange and improving production efficiencies.

During the late 1990s, there was an increase in awareness of potential negative implications of water exchange and habitat alteration caused by aquaculture. Increasingly limited water resources for a growing human global population and problems with effluent discharge from fresh water and brackishwater

aquaculture have been major concerns (Hopkins *et al.* 1995a). These concerns were not only external pressures from regulatory and nongovernmental organizations, but they increasingly arose from within the aquaculture industry itself. Examples of unregulated regional aquaculture overdevelopment demonstrated the potential negative effects of a farm receiving the effluent of neighboring farms. One of the most significant potential consequences is the concentration of pathogens and their spread from one operation to another. Disease epizootics and interest in biosecurity have been the major drivers toward adoption of reduced or zero exchange technologies for all or part of the production cycle, particularly in shrimp farming (Browdy *et al.* 1997; Lotz 1997; Stanley 2000). Adoption of low water exchange, intensive practices for land-based shrimp and tilapia culture, has yielded several distinct advantages for farmers.

- Expenses are reduced as the cost of aeration is typically lower than the cost of exchanging water, and contributions of natural productivity can reduce feed costs. Intensification and reduced water use can minimize land and water costs. For marine shrimp, reduced reliance on water exchange can provide opportunities for production in areas away from sensitive and costly coastal land.
- Operations are environmentally sustainable as water use and effluents are reduced while allowing production to be intensified, thereby reducing ecological footprints per kg product produced.
- Health is enhanced by improving biosecurity and control over introduction of pathogens while cultivating a diverse microbial community, which may improve competitive exclusion of dangerous pathogens.

With growing consumer demands for high-quality tilapia and shrimp products, increasing pressures on producers to reduce production costs, and growing efforts to assure environmental responsibility, interest in biofloc-based production technologies continues to grow.

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## 12.1 Bioflocs

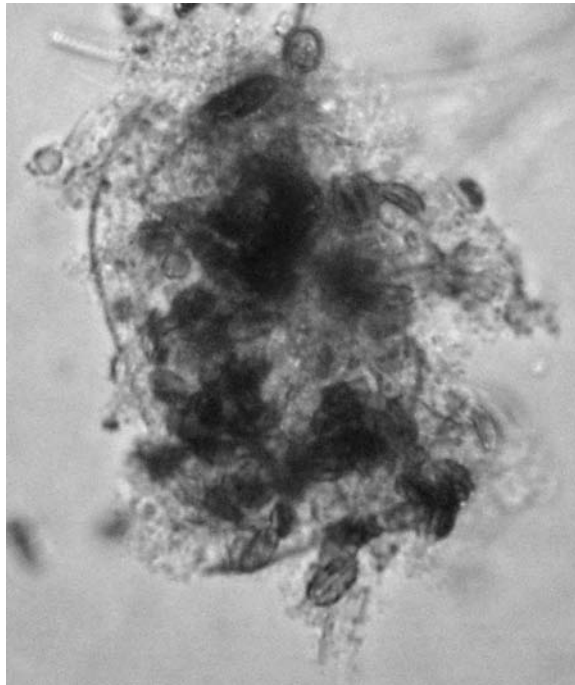
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In intensively fed aquaculture systems with reduced water exchange, high inputs of nutrients support the establishment of dense microbial communities. Fish and crustaceans use only a limited portion of the nitrogen and carbon offered in feeds for growth and metabolism. Estimates for average recovery of organic carbon, nitrogen, and phosphorus by shrimp and fish are approximately 13%, 29%, and 16% of feed content, respectively (Avnimelech & Ritvo 2003). The remainder enters the culture system either as uneaten feed or is excreted as metabolic wastes. In some recirculating aquaculture systems, the formation of a microbial community is encouraged on artificial substrates outside the animal culture area in, for example, commercially manufactured bead filters. It is this microbial community that is responsible for cycling excess nutrients. In such systems, particulate matter is often removed by external filtration such as sedimentation, vortex

devices, and sand filters. However, in biofloc systems, particles are allowed to form within the culture system and a portion of the microbial community responsible for nutrient cycling is contained within those particles.

Depending upon the intensity of feed inputs, the algal community in a system without water exchange will undergo logarithmic growth, eventually reaching a plateau due to light limitations. During this period, which can last for days in tilapia culture and up to ten weeks in shrimp systems depending on feed inputs, production performance will largely depend upon the composition of the algal community and management of associated pH and dissolved oxygen fluctuations. At this point in the production cycle, pond and tank systems typically undergo a shift from a photoautotrophically dominated community to a more bacterial dominated community. With appropriate mixing and aeration algae, bacteria, zooplankton, feed particles, and fecal matter remain suspended in the aerobic water column and naturally flocculate together, forming the particles that give biofloc culture systems their name (fig. 12.1).

These flocs are held together by physicochemical forces of attraction and a matrix of polymers composed of compounds such as polysaccharides, proteins, and humic complexes (Avnimelech 2009). The floc particles are a diverse mixture



**Figure 12.1** A biofloc particle at 100x magnification. This particle contains several algal species and likely contains multiple bacteria and fungi genera. These microbes are grazed on by zooplankton. Shrimp and tilapia may be able to consume these particles and gain nutrition from them, thereby reducing feed costs.

of microorganisms and particulate matter, and they can vary in biochemical composition and physical properties depending upon the type of feed used, target crop, type of aeration, management protocols, physical and environmental factors, and temporal and spatial variables that can change based on site, season, and factors associated with colonization (De Schryver *et al.* 2008; Ray *et al.* 2009). Biofloc communities can have fatty acid profiles distinctly unique from the feed administered to culture systems (Johnson *et al.* 2008), indicating that the microbial community is responsible for some biochemical alterations. Similar particles occur in nature and are often referred to as marine snow (Alldredge & Silver 1988). A considerable amount of research in natural systems has also been conducted on biofilm microbial communities (Costerton *et al.* 1995) and biofloc may also fall under such a description.

Bioflocs themselves represent an interesting ecosystem. The water is microbiologically a limited resources environment, having few nutrients or available organic substrates. Bioflocs are nutrient rich microenvironments embedded within nutrient-poor water. This nutrient density attracts organisms such as protozoa, nematodes, ciliates, and others that graze in or around the biofloc. The biofloc external polysaccharides (EPS) coating adsorbs detritus and free-living microorganisms, adding to its nutritious value. Bioflocs also provide the substrate required by most bacteria and can supply some refuge from predators (De Schryver *et al.* 2008). Biofloc systems contain bacteria, algae, zooplankton, fungi, and viruses. Each group contains taxa that may have positive effects and taxa that can contribute to negative consequences for fish and shrimp culture. Some bacteria, such as several species belonging to the genus *Vibrio*, are known shrimp and fish pathogens and have been historically problematic for aquaculture. Although *Vibrio* spp. occur in biofloc systems, research is still underway to determine whether they pose any risk to target crops. Other bacteria are highly beneficial to biofloc systems. Nitrifying bacteria ultimately transform toxic ammonia to the relatively nontoxic nitrate compound. Many heterotrophic bacteria can directly assimilate ammonia-nitrogen, thereby removing it from the water column. These valuable bacterial processes will be discussed in following sections.

Various forms of micro-, and occasionally macro-algae can be found in biofloc systems. Macro-algae can, at times, be seen growing near the surface or on structures in the water but is quickly grazed by the shrimp or fish if they have access to it. Micro-algae are found in biofloc particles and as free-living cells. Small chlorophytes (green algae), such as *Nanochloropsis* sp., are frequently concentrated within biofloc. It is unclear whether chlorophytes offer any nutritional value, but like most algae, they can assimilate ammonia-nitrogen to make cellular proteins and they photosynthesize in the presence of light.

Diatoms contain relatively high levels of the essential fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA; Volkman *et al.* 1989), providing a potential nutritional advantage for culture animals. Diatoms are found both in and outside of biofloc particles and have been implicated in improving shrimp growth (Ju *et al.* 2009). Other algae such as cryptophytes

and chrysophytes are seen occasionally. Algal communities seem to change in abundance and composition over time in biofloc systems and the causes of such changes are often unclear.

Potentially toxic algae can occur in any aquatic system and biofloc systems are no exception. Harmful algal bloom (HAB) taxa such as *Pfiesteria piscicida* have been identified in biofloc systems at WMC, but no negative effects on shrimp, fish, or humans were detected. These small, free-swimming heterotrophic dinoflagellates are uncommon in biofloc systems. A more common group of potentially harmful algae are the cyanobacteria, also known as blue-green algae. There is evidence that this group has hindered shrimp growth in aquaculture systems (Alonso-Rodriguez & Paez-Osuna 2003; Ray *et al.* 2009). Many of the cyanobacteria in biofloc systems such as *Synechococcus* spp. are pico-size ( $<2\ \mu\text{m}$ ) and seem to be contained primarily within biofloc particles. Removing a portion of the biofloc may increase light penetration and reduce cyanobacteria abundance, helping to select against cyanobacteria and favor more beneficial algae (Ray *et al.* 2009).

Zooplankton are an important assemblage in biofloc systems, as they consume both bacteria and algae and may then be consumed by shrimp and fish. Diverse arrays of zooplankton are found in biofloc systems. Free-swimming organisms such as ciliates and micro-flagellates can be seen feeding on biofloc particles. Rotifers and nematodes are commonly observed within the biofloc, consuming the flocculated material.

The size of biofloc particles may be important for animal nutrition, as large particles are likely more accessible to adult fish and shrimp. Most systems contain easily visible bioflocs in the range of mm fractions up to a few mm. Moss and Pruder (1995) demonstrated improved shrimp production when particles were above  $5\ \mu\text{m}$  in diameter. The size of biofloc particles can vary between culture systems. It seems that systems with more water pumping activity are prone to having smaller particles due to the severing action of pump impellers. Systems that rely more on airlift mechanisms or standard aeration systems are more likely to have larger particles. Not only might particle size affect whether animals can acquire them, but if particles are to be removed from the system their sizes may dictate what removal technique is appropriate. Settling containers can be used to remove a portion of the biofloc particles if the particles are large enough; however, foam fractionators may be required for smaller particles.

Although biofloc particles are advantageous to fish and shrimp, in the most intensive systems particle concentrations can build to very high levels. Due to intensive feed inputs, over  $1,000\ \text{mg TSS/L}$  is not uncommon if left unmanaged. It is unclear what the most beneficial concentration of biofloc particles is, but research has shown that managing the concentration is advantageous for intensive shrimp (Ray *et al.* 2010) and tilapia (Rakocy 1989) culture. As described below, high concentrations of biofloc particles imply elevated concentrations of the respiring organisms associated with them. Too much biofloc may increase the oxygen demand, increasing aeration/oxygenation costs and eventually reaching an unsafe level that is stressful to the culture animal (Beveridge *et al.* 1991). An

excessive abundance of particles may lead to gill clogging, thereby preventing adequate gas and ion exchange by the culture species (Chapman *et al.* 1987). Having too many particles can shade the water column and promote the occurrence of harmful algae while diminishing the abundance of more beneficial taxa (Brune *et al.* 2003; Hargreaves 2006). Managing biofloc concentration may reduce the age of the microbial community, promoting a younger and more nutritious assortment of organisms (Turker *et al.* 2003). Furthermore, the organisms that assimilate ammonia-nitrogen into their cellular structures must, at some point, be removed from the system or that nitrogen will return to the water and pose risk to the culture animals.

## 12.2 Oxygen dynamics

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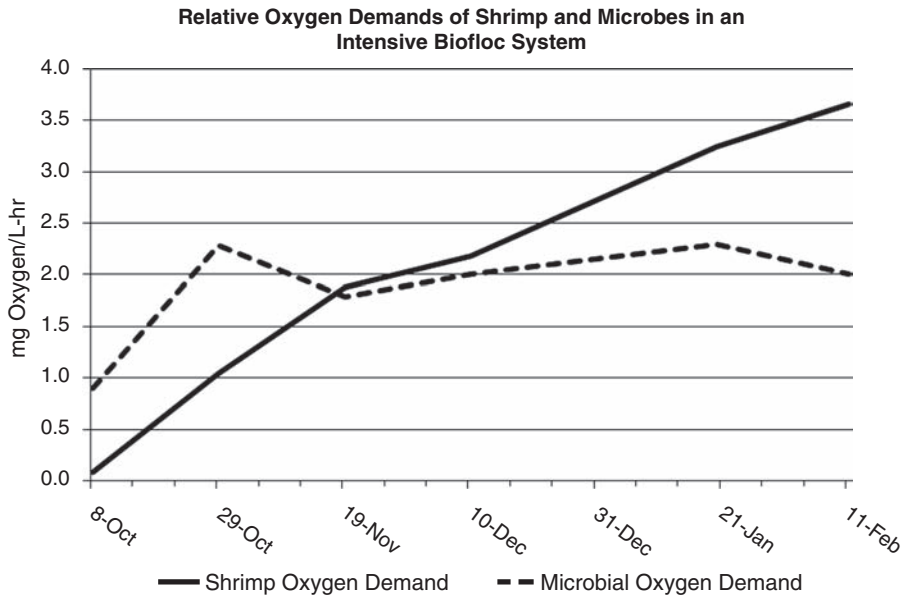
Dissolved oxygen (DO) is always one of the most critical water quality parameters to be monitored in the cultivation of aquatic organisms. Culturing organisms in biofloc systems requires that close attention especially be paid to DO. In addition to the oxygen requirements of the shrimp or fish being cultivated, the rich microbial community also consumes DO at a significant rate. The intensity of DO consumption by the microbial community is largely a function of feed inputs required for the particular stocking density (Boyd 2009). A growout study of the Pacific white shrimp, *Litopenaeus vannamei*, conducted at the WMC found that the microbial biofloc contributed significantly to the oxygen demand of the system (fig. 12.2). The shrimp stocking density was approximately 500 shrimp per m<sup>3</sup> in a greenhouse-enclosed raceway measuring 235 m<sup>3</sup>. Microbial oxygen demand exceeded shrimp oxygen demand for the first third of the growout period and still required 35 to 40% of the total oxygen demand by the end of the trial (Leffler *et al.* 2010). By the end of the growout period, the total oxygen demand for this biofloc system was approximately 5.6 mg O<sub>2</sub>/L/hr. It is essential that the system have an emergency backup system to supply oxygen in the event of loss of the primary oxygen source. Other studies at the WMC have found the microbial oxygen demand to approximately equal that of the shrimp throughout the course of the growout period. Tilapia culture in biofloc systems is associated with higher oxygen consumption by the fish, since fish biomass is in the order of 10 to 30 kg/m<sup>3</sup> (Avnimelech 2009).

The simple equation for oxygen required by cultivated organisms is that of aerobic respiration in general:



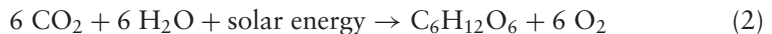
This is true of the multicellular target species as well as the single-celled bacteria, fungi, algae, and micro-invertebrates that comprise a biofloc community. Biofloc communities contain photoautotrophic, chemoautotrophic, and heterotrophic microbes. Which of these dominates depends upon the amount of organic loading entering the system through the feed inputs, other organic carbon inputs, and removal or cropping of biofloc particles from the system. At low densities,





**Figure 12.2** Relative oxygen demands of shrimp and microbes in an intensive biofloc system.

relatively little feed input will be required. This means that relatively less uneaten feed, less feces, and fewer microbes will be available to form biofloc solids. Under such conditions, given sufficient dissolved inorganic nutrients, it is possible for photosynthetic communities dominated by chlorophytes and diatoms to flourish. The abundant photoautotrophic organisms in these situations can supply considerable DO to the system during daylight hours in accordance with the general equation for photosynthesis:



The DO supplied by photosynthetic components may be sufficient to fulfill the requirements of the target species and biofloc during daylight hours, potentially remaining above critical levels through the night if stocking densities are low. However, even under these circumstances, and particularly at high stocking densities and feed loadings, DO will have to be mechanically supplied at night and during periods of prolonged cloudiness. As stocking densities and hence nutrient loading rates via the feed increase, biofloc systems become increasingly dominated by bacteria and photoautotrophs decrease in importance. According to Brune (2010), with feeding providing organic-C loading rates below 9 g C/m<sup>2</sup>-d and full sunlight, a photoautotrophic community is likely to dominate. However as organic-C loading rates exceed 12.5 g C/m<sup>2</sup>-d, biofloc algal populations decrease and the system becomes increasingly dominated by bacteria.

A great advantage of biofloc systems is the efficiency with which the microbes can process noxious nutrients released by the target species. This permits



increasingly high stocking densities resulting in potentially attractive economic results. However, as stocking densities rise and greater quantities of feed are added, the DO demand of both the target species and the biofloc microbes increases. In most pond systems for shrimp and tilapia, aeration can be provided by paddlewheel or aspirator aerators that also provide for mixing and resuspension of organic material as described further on. Aeration rates can be generally estimated based on stocking densities or calculated as a function of feeding rates. It is estimated that yield can be raised by about 375 kg for each hp aeration capacity (Boyd 2009), but reports of higher energy efficiencies up to 1,000 kg/hp are available (Avnimelech 2009).

Most commercial shrimp biofloc ponds have an area in the range of 1,000 to 20,000 m<sup>2</sup> (0.1 to 2 ha). Tilapia ponds are usually smaller, having an area of 100 to 2,000 m<sup>2</sup>. Paddle wheels and aspirator aerators are used to both aerate and mix the ponds. Aeration capacity installed is in the range of 20 to 60 hp/ha for shrimp ponds and more than 100 hp/ha in tilapia ponds where fish biomass is in the range of 200,000 kg/ha.

In shrimp tank and raceway systems, shrimp densities of about 300/m<sup>3</sup> can generally be supplied with oxygen by blowers and airstones alone. At a stocking density of 450 shrimp/m<sup>3</sup>, Samocha (personal communication) has demonstrated that a shrimp biomass load in a biofloc system can be maintained up to 7.5 kg/m<sup>3</sup> with aeration. This was the result of careful monitoring, management of biofloc particle concentration, and only occasional pure oxygen supplementation for 30 to 60 minutes after each feeding. Work at the WMC in raceway trials stocked with 1 g shrimp at 500 to 900 shrimp/m<sup>3</sup> and reusing biofloc water from previous runs, found it necessary to supply pure oxygen continuously starting as early as two weeks into the sixteen-week growout period.

The physics of oxygen transfer into recirculating aquaculture systems and the engineering approaches for achieving it have been well reviewed by Timmons and Vinci (2007). When it is necessary to introduce pure oxygen into a biofloc system, it can be supplied by an electrical oxygen generator, by compressed oxygen, or by liquid oxygen. Liquid oxygen, if available and reasonably priced, is generally preferable. For a commercial scale, high-density tank or raceway operation, liquid oxygen is more economical than compressed oxygen and can be supplied in controlled amounts as needed (e.g., immediately after feedings). An oxygen generator can be economical but requires an automated backup electrical generator in case of power failure. Oxygen can be introduced into the water through a variety of devices such as aeration cones (downflow bubble contractors), diffusion aerators (airstones), and oxygen injectors (Venturis). A Venturi design is generally economical and efficient, often taking advantage of water already being pumped for another purpose such as circulation or heating. As described below, biofloc systems must be well mixed to maintain particles in suspension. If the system is well mixed, oxygen can be injected in only a few locations and still maintain good dissolved oxygen levels throughout the system.

As shown in equation 1, for every mole of oxygen consumed through aerobic respiration, a mole of carbon dioxide is produced. Thus a super-intensive, biofloc

system that may require 5 mg O<sub>2</sub>/L/hr is also producing every hour an equal molar quantity of carbon dioxide that can accumulate in the water. Carbon dioxide is toxic to most aquacultured organisms because it reduces the capacity of blood and hemolymph to transport oxygen. While high concentrations may not be lethal to a particular species, they may impact the activity, growth rate, and disease resistance of the cultured organisms. The sensitivity of different species to dissolved CO<sub>2</sub> varies greatly and many are able to acclimate to elevated levels if the increase in concentration is gradual (Timmons & Ebeling 2007). Under low stocking densities when aeration is sufficient for maintaining oxygen, the buildup of CO<sub>2</sub> can be offset by physical degassing into the atmosphere. However, at the high animal biomass and high microbial oxygen demands that are found in super-intensive tank or raceway biofloc systems, oxygen must be injected directly into the water. Under these circumstances CO<sub>2</sub> may not be completely degassed and may accumulate to unhealthy levels. It is possible for DO levels to be sufficiently high within a system while CO<sub>2</sub> concentrations are also high. In a super-intensive shrimp raceway at the WMC, CO<sub>2</sub> levels of 80 mg/L have been measured at the same time that DO levels were maintained consistently between 6 and 7 mg/L. Because of the high metabolic activity attributable to microbes and cultured organisms, and because this demand requires direct oxygen injection, super-intensive biofloc systems may be especially prone to CO<sub>2</sub> accumulation.

Carbon dioxide dissolved in solution also affects the chemical equilibrium:



CO<sub>2</sub> production thus drives the equilibrium to the right, resulting in decreasing pH (i.e., an increase in the H<sup>+</sup> ion concentration). The decreasing pH may negatively impact the growth of the cultured species and should be monitored and chemically adjusted if necessary. Carbon dioxide accumulation both dissolved in the water and in the air above the biofloc system may become a problem especially in enclosed structures. This may cause human health concerns for workers in a poorly ventilated structure with super-intensive biofloc systems. Since CO<sub>2</sub> is heavier than air and will flow downward and away from slightly raised aquaculture containers, ventilation around the base of a greenhouse or building should allow the escape of the CO<sub>2</sub> generated by the system and reduce the buildup of gas in both the water and the air above it.

### 12.3 Resuspension, mixing, and sludge management

An intrinsic feature of aquatic systems is the sedimentation of particles from the water column to the bottom. Aquatic systems are enriched with respect to organic particles (dead algae, feed residues, etc.) at a rate proportional to the organic loading of those systems. In aquaculture, this enrichment rises with the intensity of the system. The settling of organic particles to the substrate can

create an enriched organic sediment layer, accompanied by a high sediment oxygen demand (SOD).

In ponds, diffusion of oxygen from the atmosphere through the water column and to the pond bottom is a slow process. In addition, oxygen is consumed as it diffuses down through the water. As a result of the typically high SOD and slow oxygen supply, anaerobic conditions can develop in the sediment layers of lakes, rivers, and aquaculture systems.

Anaerobic microbial processes are appreciably less efficient than those in aerobic, oxygenated systems, and thus, recycling of the residues in the sediment layer is slow compared to processes in the aerated water column. In addition, anaerobic microbial metabolism leads to the release of toxic reduced metabolites such as sulfides, reduced organic sulfur compounds, organic acids, and ammonia (Avnimelech & Ritvo 2003). The development of an anaerobic bottom layer was found to be the major limitation to increasing production in carbohydrate-fed tilapia ponds equipped with nighttime aeration (Avnimelech *et al.* 1994).

To ensure adequate nutrient cycling and further increase production, the accumulation of anaerobic bottom sludge has to be prevented. This can be achieved, as indicated in a pioneering study by Hopkins *et al.* (1994), by either removing the sludge or resuspending it. In this study, sludge removal remediated 67% of the nitrogen added to the system in feed, resulting in improved water quality. However, removal of this material leaves significant logistical and disposal problems. Discharging sludge with targeted water exchange is restricted or prohibited by environmental and biosecurity regulations and entails high pumping and/or disposal costs.

In typical recirculating aquaculture systems (RAS) the suspended residues are continuously recycled through a series of water treatment components, and the sludge is collected and dumped. An alternative method is to continually resuspend particles and prevent the buildup of anaerobic sediment, keeping the particles within the aerated water body as long as possible. This is the approach taken in biofloc systems where the organic residues are aerobically metabolized, leading to an efficient food chain and recycling, while preventing the production of toxic anaerobic metabolites. Hopkins *et al.* (1994) demonstrated that by leaving sludge in the pond and resuspending it, much of the nitrogen that entered the pond as feed could not be accounted for at the end of the production cycle, indicating volatilization from the system. It is important to note that even the existence of a few anaerobic sludge piles in the pond may affect fish or shrimp growth and water quality (Avnimelech 2009). Under anaerobic conditions hydrogen sulfide ( $\text{H}_2\text{S}$ ), which is extremely toxic, can be formed. Even low concentrations of  $\text{H}_2\text{S}$  hinder nitrite oxidation, the second stage of nitrification, thereby resulting in incidental elevated nitrite concentrations when water mixing and resuspension are not efficient.

In the previous section, aeration and oxygenation to meet metabolic demands of target crop and the microbial community were discussed. One approach to reducing the microbial oxygen demand is to aggressively crop the biofloc solids from the system. This can be accomplished in larger ponds by exchanging water

to flush solids from a central drain, or in more intensive tank systems by use of settling chambers, filtration, or foam fractionation. However, care must be exercised so as not to remove so much of the biofloc that the processes of waste assimilation and nitrification are decreased. Effective solids management has been shown to increase shrimp growth and productivity in biofloc systems (Ray *et al.* 2010a).

Aeration or supplemental oxygenation are essential to achieve super-intensive animal densities. However, aeration is also employed to achieve several related goals:

- Supply oxygen to the animals to overcome oxygen limitations and thus enable higher stocking densities, growth, and yields
- Distribute the oxygen in the system horizontally and vertically
- Mix water and sediment—water interface
- Control sludge coverage, location, and drainage

It is important that aeration should be designed, deployed, and operated so as to achieve each of these goals, as well as supply oxygen.

In pond systems, achievement of these goals depends upon proper selection and planning of aeration capacity, aerator type, aerator location, and operation mode as well as appropriate pond design. Ponds should be designed to allow for optimal drainage of sludge, between or within growing cycles. In addition, since sludge production is difficult to avoid, the pond should be designed to limit the areal coverage of sludge and sludge depth. Liners are increasingly used in biofloc-based pond systems to improve water movement and control sludge buildup while facilitating sludge oxidation and removal between crops.

The placement of aerators and types of aerators are of prime importance in managing ponds and controlling sludge accumulation, particularly when water exchange is reduced or eliminated. A very common mode of aerator placement is to locate the aerators in parallel to the dikes. This, in turn, creates a peripheral flow near the dikes and an area with no flow, or very limited flow, in the center of the pond. The physical aspects of this radial or elliptical flow were thoroughly analyzed and reported by Peterson *et al.* (2001). Qualitatively, suspended particles settling in regions with fast water movement are resuspended back into the water. However, this is not the case in the central area of radial flow. In this area, water velocity is low, or nonexistent, and settled material is not resuspended and thus settles and accumulates. A detailed analysis of this situation was conducted by Calle-Delgado *et al.* (2003). It was found that the center area of the pond was stagnant and poorly aerated. Sludge accumulated in this area and living conditions for shrimp were poor. It is important to minimize the size of the sludge pile and to design the pond for efficient sludge drainage. This can be achieved by sloping the pond bottom toward an appropriately sized drainage opening (in the center for a radial flow pattern) and by properly placing and choosing the aerators so as to mix most of the pond water.

For super-intensive tank or raceway-based systems, it is also essential that the biofloc solids remain suspended in the water column. Water within intensive

tank or raceway-based biofloc systems is moved by airstones, airlifts, pumps, or a combination of these to create either upwellings or a gentle current to maintain the particles in suspension. At very high fish or shrimp densities, bioturbation by the target crop itself contributes to biofloc resuspension.

Excessive suspended matter and pond bottom sludge must be removed since it consumes large amounts of oxygen and at very high concentrations may clog gills. Settled sludge induces anaerobic processes and can release toxic anaerobic products (Avnimelech 2009). Removal of suspended solids is practiced in super-intensive shrimp culture systems and in some cases in tilapia production tanks (Rakocy 1989; Ray *et al.* 2010b) using sedimentation tanks. In tilapia ponds, the bottom water is drained one to three times daily, stopping when the water changes from turbid to clear. In cases such as ponds in Belize Aquaculture (McIntosh 2001), bottom sludge is drained by opening a valve connecting a sump in the center of the pond to the drainage canals leading to retention ponds.

Issues related to disposal of the sludge, drained pre- or post-harvest, need attention. The sludge contains high concentrations of nutrients, reactive organic matter, and offensive reduced components. Sludge from fresh water systems can be used as a high-value soil amendment. Sludge from marine shrimp ponds on the other hand, contains salt, which precludes most land-based application. However, shrimp biosolids from a WMC pond have been used successfully as fertilizer for more salt tolerant plants like broccoli and bell peppers (Dufault & Korkmaz 2000; Dufault *et al.* 2001). Sludge can be used to produce biogas in anaerobic reactors. In cases where the pond is situated near extensive ponds, the daily drained sludge can be transferred to the extensive pond and can serve as a fish feed supplement, as practiced in Israel. More work is needed to develop methods and regulations to make sludge disposal or reuse more sustainable.

## 12.4 Nitrogenous waste products

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An intrinsic feature of intensive aquaculture systems is the buildup of inorganic nitrogen in the water. Fish and shrimp feeds contain high levels of protein (20 to 45%). Approximately 70 to 75% of the protein nitrogen in feed provided to the cultured organisms is released into the water either as uneaten feed to be broken down by microbes or as metabolic waste products. This nitrogen dissolves into the water as total ammonia nitrogen (TAN). Equilibrium is established in the water between un-ionized  $\text{NH}_3$  and ionized  $\text{NH}_4^+$  dependent upon pH, salinity, and temperature. Ammonia is lethally toxic to most organisms and even low concentrations can retard growth. Thus, its concentration must be kept as low as possible.

As an example, a fishpond holding 500 g fish/m<sup>2</sup> is fed (2% fish weight per day) with 10 g feed of 30% protein/m<sup>2</sup>-day (i.e., 3 g protein or 480 mg N/m<sup>2</sup>-day). Excretion of 75% of this amounts to about 360 mg N/m<sup>2</sup>-day. For a 1-m-deep pond this amounts to a daily build up of 0.360 mg N/L-day.

The nitrogen accumulation is ten times higher for a pond holding 5 kg fish/m<sup>2</sup>. Biofloc systems are ideally suited to deal with ammonia because the rich microbial community can take up excreted TAN rapidly and prevent its concentration from rising to dangerous levels. Three distinct groups of microbes can remove TAN through different processes: (1) assimilation by photoautotrophs (algae and cyanobacteria), (2) assimilation by heterotrophic bacteria, and (3) nitrification by chemoautotrophic bacteria. Typically, all three are present and active to varying degrees in biofloc systems depending upon stocking densities, stage of culture, system designs, and especially management strategy. As production systems are intensified, control of nitrogenous waste becomes increasingly essential.

Usually, algal dominance is found in newly stocked ponds and tanks where the concentration of substrates is too low to support bacterial dominance. Photoautotrophic biofloc systems have been used successfully to manage nitrogen wastes (Brune *et al.* 2003), but they require relatively large spatial areas and low stocking densities.

At higher feed loading rates that accompany higher stocking densities, the heterotrophic assimilation and chemoautotrophic nitrification processes will be favored. Heterotrophy becomes dominant when daily feed addition becomes high (e.g., above 450 kg feed/ha with shrimp; Chamberlain *et al.* 2001). This transition takes place much faster in ponds stocked with tilapia due to the higher biomass and feeding. The intensive growth of bacteria limits algal activity due to reduced light penetration such that only a small part of the water column receives enough light for photosynthesis. It is possible to encourage photoautotrophic activity in biofloc assemblages by controlled removal of particles to increase water clarity. During the early phase of production algae take up ammonium and convert it to protein using energy obtained from photosynthesis. Once succession occurs from an algal dominated community to a more bacterial-based system, and especially if the system is fed with carbonaceous substrates, heterotrophic bacteria have a practically unlimited capacity to assimilate the inorganic nitrogen to build microbial proteins.

Heterotrophic assimilation is an important process in most biofloc systems. Heterotrophic bacteria and other microorganisms use carbohydrates (sugars, starch, and cellulose) as a food to generate energy and to grow:



The percentage of the assimilated carbon with respect to the metabolized feed carbon is defined as the microbial conversion efficiency (E) and is in the range of 40 to 60%. Nitrogen is required as an important building block of the microbial cell. Thus, microbial utilization of organic carbon is accompanied by the assimilation of inorganic nitrogen. This is a basic microbial process and practically all microbial assemblages perform it.

The amount of carbohydrate supplement ( $\Delta\text{CH}$ ) required to reduce the ammonium can be calculated (Avnimelech 1999). Based on equation 4 and the definition of the microbial conversion coefficient, E, the potential amount of

microbial carbon assimilation when a given amount of carbohydrate is metabolized ( $\Delta\text{CH}$ ) is:

$$\Delta\text{C}_{\text{mic}} = \Delta\text{CH} \times \%C \times E \quad (5)$$

Where  $\Delta\text{C}_{\text{mic}}$  is the amount of carbon assimilated by microorganisms and  $\%C$  is the carbon contents of the added carbohydrate (roughly 50% for most substrates). The amount of nitrogen needed for the production of new cell material ( $\Delta\text{N}$ ) depends on the C/N ratio in the microbial biomass, which is about 4.

$$\Delta\text{N} = \Delta\text{C}_{\text{mic}}/[\text{C/N}]_{\text{mic}} = \Delta\text{CH} \times \%C \times E/[\text{C/N}]_{\text{mic}} \quad (6)$$

And using approximate values of  $\%C$ ,  $E$ , and  $[\text{C/N}]_{\text{mic}}$  as 0.5, 0.4, and 4, respectively:

$$\Delta\text{CH} = \Delta\text{N}/(0.5 \times 0.4/4) = \Delta\text{N}/0.05 \quad (7)$$

According to equation 4 (assuming that the added carbohydrate contains 50% C), the CH addition needed to reduce TAN concentration by 1 mg/L N (i.e., 1g N/m<sup>3</sup>) is 20 mg (20 g/m<sup>3</sup>). This relationship enables a manager finding a high TAN concentration in the pond (following cloudy days, an algae crash, high animal biomass, etc.), to calculate how much carbohydrate substrate must be added to mitigate an otherwise dangerous situation. This mode of action may be considered an emergency, post factum mode. The manager reacts following the excessive rise of TAN or NO<sub>2</sub>. While heterotrophic bacteria do not efficiently assimilate NO<sub>2</sub>, the carbohydrate stimulation causes them to take up TAN, leaving less for chemoautotrophic bacteria to convert to NO<sub>2</sub>.

A different, proactive approach is to add the right amounts of carbohydrate with the feed in order to prevent unwanted TAN increase and to optimize the process. Here, one has to estimate the amount of carbohydrate that has to be added in order to immobilize the ammonia excreted by the fish or the shrimp in real time. As mentioned, fish or shrimp in the pond assimilate only about 25% of the nitrogen added in the feed. The rest is excreted mostly as TAN (some as organic N in feces or feed residue). It can be assumed that the TAN flux into the water,  $\Delta\text{TAN}$ , or generally  $\Delta\text{N}$ , directly by excretion or indirectly by microbial degradation of the organic N residues, is at least 50% of the feed nitrogen flux:

$$\Delta\text{N} = \text{Feed} \times \%N_{\text{feed}} \times \%N_{\text{excretion}} \quad (8)$$

A partial water exchange, sedimentation, or removal of sludge reduces the TAN flux in a manner that can be calculated or estimated. The amount of carbohydrate addition needed to assimilate the TAN flux into microbial proteins is calculated using equations 7 and 8:

$$\Delta\text{CH(g)} = [\text{Feed(g)} \times \%N_{\text{feed}} \times \%N_{\text{excretion}}]/0.05 \quad (9)$$



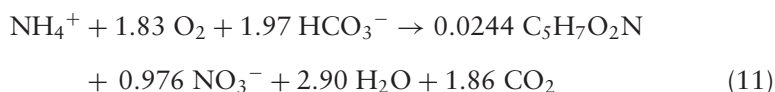
For example: Assuming 30% protein feed pellets (4.65% N) and assuming that 50% of the feed nitrogen is excreted (%N excretion), we get:

$$\Delta\text{CH(g)} = \text{Feed(g)} \times 0.0465 \times 0.5/0.05 = 0.465 \times \text{Feed(g)} \quad (10)$$

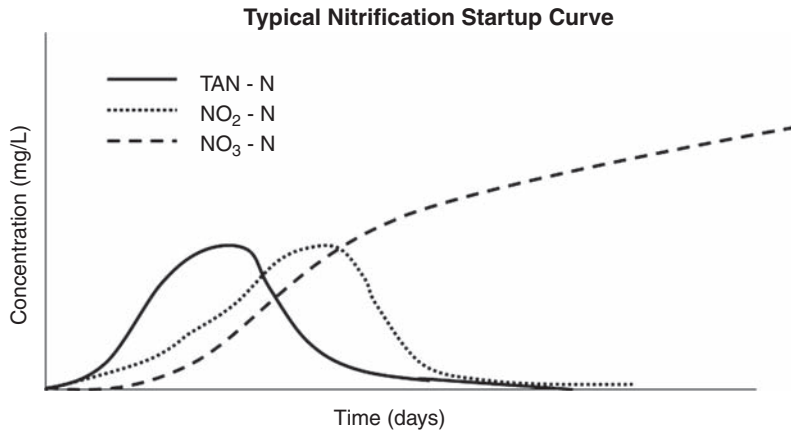
According to equation 10, the feed having 30% protein should be amended by an additional portion of straight carbohydrates amounting to 46.5% addition to the feed ration.

Another means of controlling TAN is to rely on nitrification. Nitrification is a two-step process in which one group of bacteria oxidizes TAN as its primary energy source, consuming bicarbonate ions as its carbon source, and producing nitrite ions as a by-product. Ammonia-oxidizing bacteria include the genera *Nitrosomonas*, *Nitrosococcus*, *Nitrospira*, *Nitrosolobus*, and *Nitrosovibrio* (Timmons & Ebeling 2007). Nitrite produced by this process can be more toxic than ammonia, altering the hemoglobin molecule to prevent oxygen uptake (Tomasso *et al.* 1979). A second group of bacteria oxidizes nitrite to nitrate to obtain energy, again consuming bicarbonate ions and dissolved carbon dioxide as carbon sources. These bacteria typically belong to the genera *Nitrobacter*, *Nitrococcus*, *Nitrospira*, and *Nitrospina* (Timmons & Ebeling 2007). Both groups of these chemosynthetic bacteria are obligate autotrophs and obligate aerobes. Typically, when a new biofloc system is started it will take some time for the nitrifying bacteria to become established. Because nitrite is produced as a product of the first step, there will typically be a lag between establishing the ammonia-oxidizing population and the nitrite-oxidizing population. Nitrate is produced as a product of the second process. It is relatively harmless at concentrations into the hundreds of mg/L and continues to accumulate in the system as nitrification proceeds. A typical example of the dynamics of the nitrogen compounds involved in nitrification is depicted in figure 12.3. As described previously, during the early ammonia and nitrite accumulation periods, dextrose or another simple carbohydrate source can be added to a biofloc system to dampen the spikes of these compounds. Stoichiometry of carbohydrate addition to stimulate the heterotrophic bacterial population has been discussed above. At the WMC, dextrose is used routinely to control ammonia and nitrite spikes in shrimp biofloc systems until the nitrification process becomes fully functional.

Both the ammonia-oxidizing and the nitrite-oxidizing steps of the nitrification process can be represented by the combined equation (Ebeling & Timmons 2007):



As TAN is removed from the system to provide energy for the bacteria, oxygen and bicarbonate ions are also consumed. As a result, nitrifying bacteria contribute significantly to the oxygen demand on the system and especially to the consumption of alkalinity. In order to maintain the nitrification process as well



**Figure 12.3** Typical nitrification startup curves.

as buffer against decreasing pH, alkalinity, generally in the form of  $\text{NaHCO}_3$ , must be added on a regular basis. Approximately 7.05 g of  $\text{HCO}_3^-$  is required for every g N removed in order to maintain alkalinity. How much total alkalinity is required is a direct function of the nitrogen loading rate from the feed used to support a given stocking density. The nitrification process also produces nitrate and carbon dioxide. Nitrification is evident in a biofloc system if nitrate steadily increases throughout a production cycle (fig. 12.3). This is distinct from photoautotrophic and heterotrophic assimilation processes in which nitrate levels remain unchanged or decrease. Carbon dioxide production contributes to the  $\text{CO}_2$  that is also generated by aerobic respiration.

These two management strategies each have advantages and disadvantages and may be more or less suitable to different biofloc production systems depending upon target species, stocking density, and so on. It is important to note that both nitrogen assimilation and nitrification take place in all biofloc systems. The difference among systems is the rate of use of added carbohydrates (or the equivalent use of low protein feed), opposed to use of carbohydrate additions only occasionally when TAN is too high. Table 12.1 compares the implications for water quality and economic considerations that result from the removal of 1 g of ammonia-nitrogen by the mechanisms of nitrification and heterotrophic assimilation.

Heterotrophic assimilation requires the addition of significant amounts of carbohydrate—approximately 15 g of carbohydrate for every gram of TAN removed (table 12.1). Since nitrifying bacteria obtain their energy from oxidizing TAN, no additional organic carbon is required to drive their metabolism. Although simple carbohydrates are relatively inexpensive, the quantities required may become an increasingly significant cost at higher stocking densities. Costs of added carbohydrates are partially offset in some intensive systems by the contribution of the flocs directly to the nutrition of the target crop resulting

**Table 12.1** Comparison of stoichiometric balances for the removal of 1 g of ammonia-nitrogen by the mechanisms of nitrification and heterotrophic assimilation (from Ebeling et al. 2006).

per g of NH <sub>3</sub> -N consumed	Nitrification (g)	Heterotrophic assimilation (g)
Carbohydrate consumed	0	15.17
Alkalinity consumed	7.05	3.57
Oxygen consumed	4.18	4.71
Bacterial Biomass produced	0.20	8.07
CO <sub>2</sub> produced	5.85	9.65
NO <sub>3</sub> -N produced	0.976	0

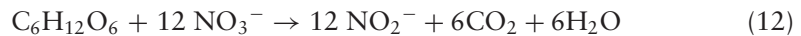
in improved protein utilization. Nitrification consumes more alkalinity than heterotrophic metabolism, which requires bicarbonate ions to be added to the system, usually in the form of NaHCO<sub>3</sub>, in order to maintain pH and support continued functioning of both mechanisms. Depending on feeding rates, the use of aerators and high CO<sub>2</sub> volatilization can offset the drop of alkalinity in many pond systems. Nitrification can also be slow to establish, resulting in the accumulation of intermediate products and the production of nitrate, which in intensive systems with complete water reuse between cycles, could eventually become problematic. Both processes require approximately equal amounts of oxygen; however, heterotrophic assimilation produces 65% more carbon dioxide per gram of TAN, which can be a significant problem in enclosed systems stocked at high densities.

A substantial difference between these two processes, however, is the quantity of solids produced by heterotrophic assimilation, approximately forty times that of nitrification. Driven by the large input of carbohydrates, assimilation sequesters TAN into bacterial biomass. If left in the system, a portion of the bacteria may be consumed by the culture species; the rest eventually die and release the TAN back into the water. Therefore, as stocking densities rise, the heterotrophic assimilation process must be coupled with a solids removal mechanism. This requires both additional effort and expense, and, once removed, disposal of the solids must be addressed. Nitrification produces relatively minor amounts of solids. Instead, it converts TAN ammonia-nitrogen into nitrate that is harmless to most cultured species even in relatively high concentrations. Where nitrate levels may possibly accumulate to levels of concern would occur in situations where the biofloc water is retained and reused for multiple growout runs that are stocked at very high densities.

In super-intensive shrimp biofloc raceway systems at WMC, nitrification is the dominant mechanism for effectively removing TAN even at stocking densities as high as 900 shrimp/m<sup>3</sup>. Carbohydrate in the form of dextrose is only added as necessary at the start of a trial with new water to suppress initial ammonia and nitrite spikes while the nitrifying bacteria are becoming established. Once these populations are established, there are typically no further problems with ammonia and nitrite. When the biofloc water is retained and reused for the

next growout run, no significant ammonia or nitrite spikes are usually observed. Nitrate levels increase during the course of each growout, although not as much as would be expected if nitrification was solely responsible for TAN remediation, based on nitrogen entering a system through feed. A nitrogen mass balance study conducted at WMC involved thirty-two outdoor tanks stocked at different densities with different levels of solids removal and different levels of nitrogen inputs. No carbohydrate was added to stimulate assimilation. Shrimp nitrogen conversion efficiencies averaged  $27 \pm 2\%$  (mean  $\pm$  SE). Cropping of solids reduced nitrate levels 18 to 44% depending on treatment. However,  $29 \pm 4\%$  of nitrogen introduced to the tanks left the systems as volatilized ammonia or as nitrogen gas produced through denitrification or other reduction processes.

Under anaerobic conditions nitrate ( $\text{NO}_3^-$ ) can be reduced to nitrogen gas ( $\text{N}_2$ ) through denitrification, resulting in complete removal of nitrogen from the system. Denitrification is conducted by heterotrophic bacteria capable of utilizing  $\text{NO}_3^-$  as an electron receptor in the absence of  $\text{O}_2$  leading first to the production of nitrite ( $\text{NO}_2^-$ ) and ultimately to the production of  $\text{N}_2$  as a byproduct (Wetzel 2001).



Biofloc systems are kept well aerated and mixed to avoid the development of anaerobic accumulations of organic material that might release toxic sulfides and other compounds. However, it has been speculated that denitrification might occur at significant levels in the oxygen-depleted interior of the biofloc particles suspended within the water column. This is a reasonable hypothesis to explain the observation that nitrate generally does not accumulate at the rate predicted by a simple mass balance of nitrogen inputs into a biofloc system. The anammox pathway might also occur in the anaerobic interiors of biofloc particles. This process bypasses a portion of the denitrification reaction by directly converting ammonia and nitrite to  $\text{N}_2$  gas. If present, the anammox reaction and the denitrification process are probably complementary and occur simultaneously (Tal *et al.* 2009).

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## 12.5 Temperature

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Effects of temperature on bioloc-based systems are similar to those in traditional culture units. The greatest effect of temperature is its relationship with growth rate. In any aquaculture system, temperature is correlated with growth performance, as all cultured animal species are poikilothermic. This limitation constrains growing season in some areas, affecting the number and length of crop cycles in shrimp and the ability to overwinter tilapia. In tropical regions with high temperatures year round, the temperature in biofloc systems is similar

to that of traditional systems. However, as one moves to more marginal temperate zones, water exchange can significantly affect temperatures. Thus, ability to culture without exchange has the advantage of allowing for more stable thermal conditions. In shrimp culture, one of the most virulent viral diseases, white spot syndrome virus, has been shown to be highly temperature dependent (Vidal *et al.* 2001). Subsequent to this discovery, management strategies for this disease include maintaining pond temperatures at 29°C and above. These strategies often necessitate avoiding water exchange and even enclosure of production ponds in greenhouses. Use of biofloc technologies allows growers to maintain pond temperatures by eliminating the need for water exchange.

As described above, continuing intensification of shrimp production in biofloc-based systems has resulted in tank and raceway culture systems that have reached production levels exceeding the equivalent of 100,000 kg/ha/crop in experimental systems (Otoshi *et al.* 2007). At these production levels, experimental systems are enclosed in greenhouses, which allow better control of temperature and improved biosecurity (Browdy *et al.* 2009). Li *et al.* (2009) modeled this type of system demonstrating significant gains in heat retention and providing a model to calculate supplemental energy needs to run these types of systems year round in various geographic locations. As discussed below, financial models then can be used to evaluate opportunities for year-round shrimp culture based on biofloc systems in temperate zones close to large markets (Hanson *et al.* 2009).

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## 12.6 Feeds and feeding

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Feeds and feeding are critical to any aquaculture operation. Feeds are the most significant part of variable costs of operating a biofloc system (Hanson *et al.* 2009) and feed performance is intimately related with target crop production success both in terms of direct nutrition and in terms of effects on water quality. Recent reviews have been published on current trends in shrimp and tilapia nutrition (Li *et al.* 2006; Lim & Webster 2006; Davis & Sookying 2009; Hardy 2009). Research has focused on efforts to reduce fish meal content in diets, as fish meal represents a limited resource with increasingly volatile price fluctuations. One of the most important trends in aquaculture nutrition research today is to explore the direct and indirect effects of inclusion of alternative protein sources in aquaculture diets. These effects can include changes in nutrient composition and availability, effects on gut health and gut microbial flora, problems with anti-nutritional factors, etc. When culturing animals in biofloc systems, the natural productivity within the system can make important contributions to nutritional balance. Numerous references indicate the growth-enhancing effect of pond water in general and of culturing fish and shrimp in biofloc systems in particular (Leber & Pruder 1988; Moss *et al.* 1992; Wasielesky *et al.* 2006). Burford *et al.* (2004), Bianchi *et al.* (1990), Avnimelech (2007), and Avnimelech and Kochba (2009) demonstrated for both shrimp and tilapia direct biofloc consumption using stable isotope techniques. Biofloc fatty acid composition has been documented at levels comparable to those found in commercial feeds (Tacon *et al.*

2002). Moss *et al.* (2006) showed that shrimp could be grown in biofloc systems using feeds without supplemental vitamins without significantly reducing growth, and flocs have been shown to contain important minerals and amino acids (Avnimelech 2006; Tacon *et al.* 2002). Thus, biofloc can provide important essential nutrients either to enhance performance when using a complete feed or to allow new directions for reduced cost formulations by taking advantage of floc nutritional contributions. Burford *et al.* (2004) showed that nitrogen conversion efficiencies from feed sources might be increased 18 to 29% in shrimp biofloc-based systems. Avnimelech showed increased protein uptake efficiency in tilapia from 25% to about 50% in biofloc systems. The assimilation of waste nitrogen by the microbial biofloc and reingestion by the target crop can significantly enhance conversion efficiencies, improving environmental sustainability and potentially increasing profitability.

In addition to affecting shrimp or fish growth, variations in protein source or protein levels affect digestibility of the feed and hence feed utilization. In biofloc systems these factors take on increasing importance as waste material is either mineralized or assimilated within the system. Clearly, as stocking densities increase above the most extensive levels, feed inputs become the most important drivers of processes within the system. As discussed above, meeting oxygen demand both by target crops and by the microbial community in biofloc systems is one of the most important aspects of systems management.

Models have been developed demonstrating the direct relationships between feed inputs and aeration requirements in shrimp production systems (Hopkins *et al.* 1991; Boyd 2009). Efficiency of feed utilization has a direct effect on biofloc density, microbial oxygen demand, and sludge production. Two strategies can be found in the literature for managing feed inputs in biofloc systems. One strategy focuses on use of nutrient dense high-protein feeds with highly digestible ingredients offered with feeding strategies, which emphasize control of feed conversion ratios (Kureshy & Davis 2002; Browdy *et al.* 2009). The goal is to provide just enough feed to remain slightly below maximum target crop demand. A second strategy suggests addition of lower protein feeds or mixtures with grain-based supplements to encourage heterotrophic biofloc production and assimilation of waste (Avnimelech 1999; McIntosh 2001; Ebeling *et al.* 2006). Selecting a feed formulation strategy can depend upon the particulars of the biofloc system. For example, when rearing shrimp in ponds at lower densities in low salinity systems, exigencies of ammonia and nitrite toxicity can outweigh dangers from high biofloc densities as long as buildup of anoxic organic material is prevented. In this case, lower protein feeds or higher carbon inputs should be considered. On the other hand, as shrimp rearing densities are increased in higher salinity systems, controlling biofloc densities and managing waste buildup is critically important. In this case, with greater system resiliency in terms of ammonia and nitrite toxicity levels, due to higher salinity, and greater reliance on nitrification to control ammonia and nitrite levels, higher protein feeds and emphasis on feed utilization efficiencies can result in fast target crop growth and minimal waste production. Similarly, culture and feeding of tilapia can be quite different from that of shrimp, as the fish more efficiently consume

particles from the biofloc in the water column. For any specific application of the technology, applying an understanding of the dynamic relationships between feeding behavior and feed utilization efficiencies and target crop and microbial community oxygen demand is critical to designing and managing biofloc system efficiencies, particularly at higher densities.

## 12.7 Economics

Biofloc-based systems can have significant advantages in terms of economic returns when compared to conventional systems, which rely on water exchange. In terms of variable costs, as early as the mid-1990s it was suggested that aerating ponds can be more cost effective than pumping water to maintain dissolved oxygen levels (Hopkins *et al.* 1995b). Use of aeration also allows for increased stocking densities and intensification of production. Perhaps the most important driver of profitability in shrimp culture is maintaining a high survival rate. In sensitivity analyses of shrimp culture in earthen ponds in Texas, it has been estimated that 90.9% of total variation can be assumed by variation in survival (Moss & Leung 2006). Thus it is not surprising that the major driver of conversion to biofloc technologies is disease control. In most shrimp culture systems, management strategies prioritize reducing losses from opportunistic bacterial infections or from excludable pathogens such as viruses. The reduction of water exchange enabled by biofloc technologies and more diverse microbial communities in well-managed biofloc systems, enables biosecurity and reduces opportunities for dominance by harmful microbes, thus driving the system toward more stable survival.

Growth rate and feed conversion efficiency also play important roles in total cost variations in both pond-based and intensive tank or raceway-based shrimp culture systems, representing 23.1 and 19.6%, respectively, for systems analyzed in the United States (Moss & Leung 2006). These authors found that, based on more stable survivability estimates and the harvest of multiple crops per year, a hypothetical super-intensive system for culture of shrimp in biofloc-based raceway systems was found to be a more economically viable alternative than earthen pond culture of penaeid shrimp in the United States. In most major shrimp-producing countries, feed represents the highest operating cost item ranging from a low of 25% to a high of 45% of total operating costs (Tacon *et al.* 2006). Opportunities to better control feed usage and improve feed conversion ratios represent a key component in controlling variable costs. It remains to be seen if feed formulations can be modified to reduce feed costs by leveraging contributions from natural productivity in biofloc systems without affecting long-term survival and growth.

In an analysis of an economic model for super-intensive biofloc tank-based shrimp production Hanson *et al.* (2009) compared the effects of varying biological parameters on net present value (NPV) and internal rate of return (IRR). These authors found that the greatest effects on cost efficiency and returns came from improvements in survival (20% improvement increased IRR by 97%). A



20% increase in stocking densities or growth rate increased NPV by 57 and 45%, respectively. On the other hand, a 20% decrease in feed cost or cost of post-larvae only reduced NPV by 22 and 9%, respectively. Clearly, investments in infrastructure, seed, and feed that can improve survival, growth rate, and stocking densities can have strong, positive impacts on economic returns.

## 12.8 Sustainability

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Sustainable development requires consideration of environmental resource management, social factors, and economic factors. In planning for future sustainable aquaculture development, continued growth, and expansion of the sector must be taken into account. According to FAO projections, there is a need to increase aquaculture production fivefold by the year 2050. This huge expansion must be accomplished in a sustainable way. However, until recently, aquaculture development planning did not include enough sustainability considerations, especially during the period of 1980 to 2000, when shrimp production was considered to be highly profitable and new shrimp ponds were constructed, in some cases with little regard for the environment. The expansion of shrimp ponds in some areas resulted in mangrove destruction; the unregulated release of effluents into rivers, lakes, estuaries, and enclosed marine regions; and the eutrophication of receiving bodies. These developments led to harsh objections from environmental groups and concerned scientists, opposing further unsustainable development and eventually leading to increased environmental regulations and development of certification programs based on best management practices.

A major issue related to the effect of aquaculture on the environment is the release of pollutants with the drained water. Culture systems are fed and often fertilized, leading to relatively high concentrations of nutrients such as nitrogen and phosphorus. Emissions of water into receiving bodies may raise nitrogen and phosphorus concentrations to levels that endanger water quality. Most aquaculture systems also contain high concentrations of organic matter, both soluble and particulate. These compounds are biologically degradable, consuming oxygen as they break down, and often causing anoxic or hypoxic conditions in the water and sediments around the effluent discharge. Concerns have also been raised regarding emissions of pathogenic microorganisms that may impact the natural biota in the receiving water, especially if the pathogens emitted are not normally found in the receiving water. In such a case, the natural biota could have a low resistance toward those pathogens (e.g., Hopkins *et al.* 1995a). Traditional aquaculture systems may exchange a high percentage of their water with the environment and thus potentially endanger the surrounding environmental quality. A production system that does not release large volumes of water to the environment is, in this respect, a potentially more environmentally friendly system.

Another aspect of aquaculture sustainability is the optimal utilization of natural resources, mostly land and water. FAO predicts a fivefold increase in aquaculture production but there is not enough water and land available to raise

production fivefold using traditional techniques. A conventional pond, producing 2,000 kg of fish/ha and losing 35,000 m<sup>3</sup> of water per year by evaporation and seepage—and an additional 10,000 m<sup>3</sup> with the water drainage—will consume 45 m<sup>3</sup> of water to produce 1 kg fish, and the production rate per unit area will be 0.2 kg/m<sup>2</sup>. In contrast, the water consumption in intensive, zero, or limited water exchange systems is less than 1 m<sup>3</sup> per kg fish production and the productivity of the pond area is in the range of 10 to 100 kg fish/m<sup>2</sup>. Using such systems relieves water or land limitations to further production of fish, making more optimal usage of land and water.

An additional environmental issue that can constrain future aquaculture development and reduce economic viability is the dependence of aquaculture on fish products originating in the marine environment. Fish meal and fish oils are common components of fish feed. Marine finfish feeds were traditionally made of about 50% fish meal, marine shrimp feeds contained about 30% fish meal, and tilapia feeds about 15%. According to estimates published by Naylor *et al.* (2000), approximately 5 kg wild fish were needed at that time to produce 1 kg of carnivorous marine fish (Naylor *et al.* 2000). However, much less (<1 kg) is needed to produce omnivorous carps. The dependence upon wild fish for feeds is potentially an important factor endangering marine ecology due to the degradation of fish populations in many marine regions. Furthermore, uncontrolled use of marine fish in aquaculture diets may introduce marine contaminants such as mercury and PCBs to the culture organism. A replacement of this protein source by a more sustainable feeding program is vital for the development of aquaculture. Many efforts have been made in order to develop fish feeds in which plant proteins replace wild fish sources. The improvements in protein utilization demonstrated in biofloc-based systems and potential for further increases in utilization efficiencies represents a major contribution toward the sustainability of aquaculture (Avnimelech 2009). Further improvement of feed quality in bioflocs as previously discussed is a topic worthy of further research.

An environmental issue common to all intensive aquaculture systems is the proper treatment and disposal of accumulated sludge. Sludge is the term for solids removed from the aquaculture system representing a concentrated source of reactive organic matter, rich in nutrients, similar to sludge produced in wastewater treatment systems (without the human pathogen risks). Sludge originating from wastewater treatment plants is usually treated by incineration or by anaerobic processes such as fermentation (producing bio-gas). Sludge is then further treated by composting (with potential agriculture uses depending upon salt content). These treatments add significantly to the cost of wastewater treatment. Presently, there are no well-established methods for treating aquaculture sludge. However, there are some studies and practical experiences of using such sludge as a source of feed for shrimp and fish (Kuhn *et al.* 2008; Schneider *et al.* 2006), as a feed applied to adjacent extensive ponds, or as a soil amendment. An important point that needs further study and quantitative data analysis is the fact that in biofloc systems, residues are suspended over long retention times in aerobic sections of the pond and can be utilized, to a large extent, by culture species like tilapia. These factors probably lead to a better degradation and possibly lower

amounts of sludge accumulation compared with typical recirculating aquaculture systems, or possibly lower amounts per kg fish as compared to semi-intensive ponds (Hopkins *et al.* 1993). The problems of sludge minimization, utilization, and handling will likely be the subject of increasing regulation and should be studied further.

## 12.9 Outlook and research needs

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The expanding application of the principles of biofloc technology in many parts of the world have reinforced the potential advantages inherent in this type of system while raising more questions and research needs to overcome problems and enhance efficiencies. Perhaps the most important area of need is an increased understanding of the complex biofloc microbial communities and developing management techniques to direct and optimize their establishment, stability, and control of structure and activity. This is closely related to issues surrounding water use in the system and potential for reuse of water within and among production units. Additional research is needed to better understand factors affecting sludge production and management in biofloc systems so as to maximize efficiency of feed conversion to fish or shrimp flesh while minimizing waste production. Engineering and design of pond and tank systems are important areas of research in this regard, particularly in the context of improving energy efficiencies and reducing carbon footprints. Genetic selection programs have produced improved strains of tilapia and shrimp, demonstrating the potential for increased growth and more robust stocks. Selection of stocks for traits that enhance performance in biofloc-based systems could represent a significant opportunity. As previously mentioned, development of specialty feeds and improvement of feed management are high priorities both because of their impacts on economics and because of effects on water quality and microbial community management. Continued development and application of bioeconomic models and establishment of key production metrics related to energy, water, and other resource uses will also help to focus research efforts, improving system efficiencies and sustainability both from environmental and financial perspectives.

This review and other literature on the topic of biofloc systems tend to focus on the generalities between various system types, levels of intensity, scales of operations, and target species. Clearly, there are important differences between the culture of tilapia and that of shrimp in terms of systems management, biomass density, associated feeding rates, feed composition, and the biology of the species, particularly in terms of their ability to crop biofloc directly from the system. Similarly, system differences between intensive ponds and super-intensive tank and raceway culture systems can lead to important differences in philosophy in terms of feed nutrient densities, strategies for management of nitrogenous wastes, and cropping of excess organic material from the system. When considering the commercial application of these technologies it is important to carefully consider these factors as design and management strategies are developed.

The future outlook for the continued adoption and expansion of biofloc-based production technologies is bright, representing important opportunities to concurrently expand environmental sustainability while opening new options for reducing production costs and improving consistency and profitability.

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